Rickettsial Infections of Fleas Collected From Small Mammals on Four Islands in Indonesia

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ABSTRACT Ectoparasites were sampled from small mammals collected in West Java, West Sumatra, North Sulawesi, and East Kalimantan, Indonesia, in 2007–2008 and were screened for evidence of infection from bacteria in the Rickettsaceae family. During eight trap nights at eight sites, 208 fleas were collected from 96 of 507 small mammals trapped from four orders (379 Rodentia; 123 Soricomorpha; two Carnivora; three Scandentia). Two species of fleas were collected: *Xenopsylla cheopis* ($n = 204$) and *Nosopsyllus* spp. ($n = 4$). Among the 208 fleas collected, 171 *X. cheopis* were removed from rats (*Rattus* spp.) and 33 *X. cheopis* from shrews (*Suncus murinus*). *X. cheopis* were pooled and tested for DNA from rickettsial agents *Rickettsia typhi*, *Rickettsia felis*, and spotted fever group rickettsiae. *R. typhi*, the agent of murine typhus, was detected in *X. cheopis* collected from small mammals in West Java and East Kalimantan. *R. felis* was detected in *X. cheopis* collected from small mammals in Manado, North Sulawesi. *R. felis* and spotted fever group rickettsiae were detected in a pool of *X. cheopis* collected from an animal in East Kalimantan. Sixteen percent of the *X. cheopis* pools were found positive for *Rickettsia* spp.; four (10.8%) *R. typhi*, one (2.7%) *R. felis*, and one (2.7%) codetection of *R. felis* and a spotted fever group rickettsia. These data suggest that rickettsial infections remain a threat to human health across Indonesia.

KEY WORDS *Xenopsylla cheopis*, rickettsia, small mammals, Indonesia

Emerging and re-emerging infectious diseases continue to contribute to morbidity and mortality in developing nations (Azad et al. 1997). Rickettsial diseases are endemic in Indonesia, but because of lack of diagnosis and agent-specific diagnostic assays, the disease burden is unknown across the archipelago (Richards et al. 1995). These pathogens are zoonotic (the vertebrate reservoir is usually rodent species) and are spread to humans by infected ectoparasites (invertebrate hosts). *Xenopsylla cheopis*, an important plague vector, is also a vector of several rickettsial pathogens (Traub et al. 1978, Azad and Traub 1989, Jiang et al. 2006). Murine typhus (also known as endemic typhus and fleaborne typhus), caused by *Rickettsia typhi*, is found worldwide. Indonesia has one of the highest prevalence levels of antibodies to *R. typhi* among people in the world (Richards et al. 2002). Studies in Java found that people living in urban areas had a higher prevalence of antibodies to *R. typhi* than less urban areas with lower concentrations of people (Richards et al. 2002). Flea-borne spotted fever (cat flea typhus) caused by *Rickettsia felis* has been identified in *Ctenocephalides felis* found in North America, Europe, Africa, Asia, Australia, and New Zealand and in *X. cheopis* in Java, Indonesia (Jiang et al. 2006). Scrub typhus (*Orientia tsutsugamushi*) has been recognized in Indonesia since World War II, where it was a major nonbattle injury for military forces (Griffiths 1945). Richards et al. (2003) found evidence of spotted fever group (SFG) rickettsiae (SFGR) infection in human residents of Gag Island, Indonesia, located northwest of the island of Irian Jaya in Eastern Indonesia.

To determine the presence and prevalence of rickettsia pathogens in ectoparasites, we surveyed live captured, small mammals trapped in lowland and highland villages on four islands in Indonesia. In this study, we report results from a survey of *X. cheopis* found on small mammals in four islands of Indonesia, Java, Sumatra, Sulawesi, and Kalimantan to determine the distribution of flea-borne rickettsial agents.

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14. ABSTRACT

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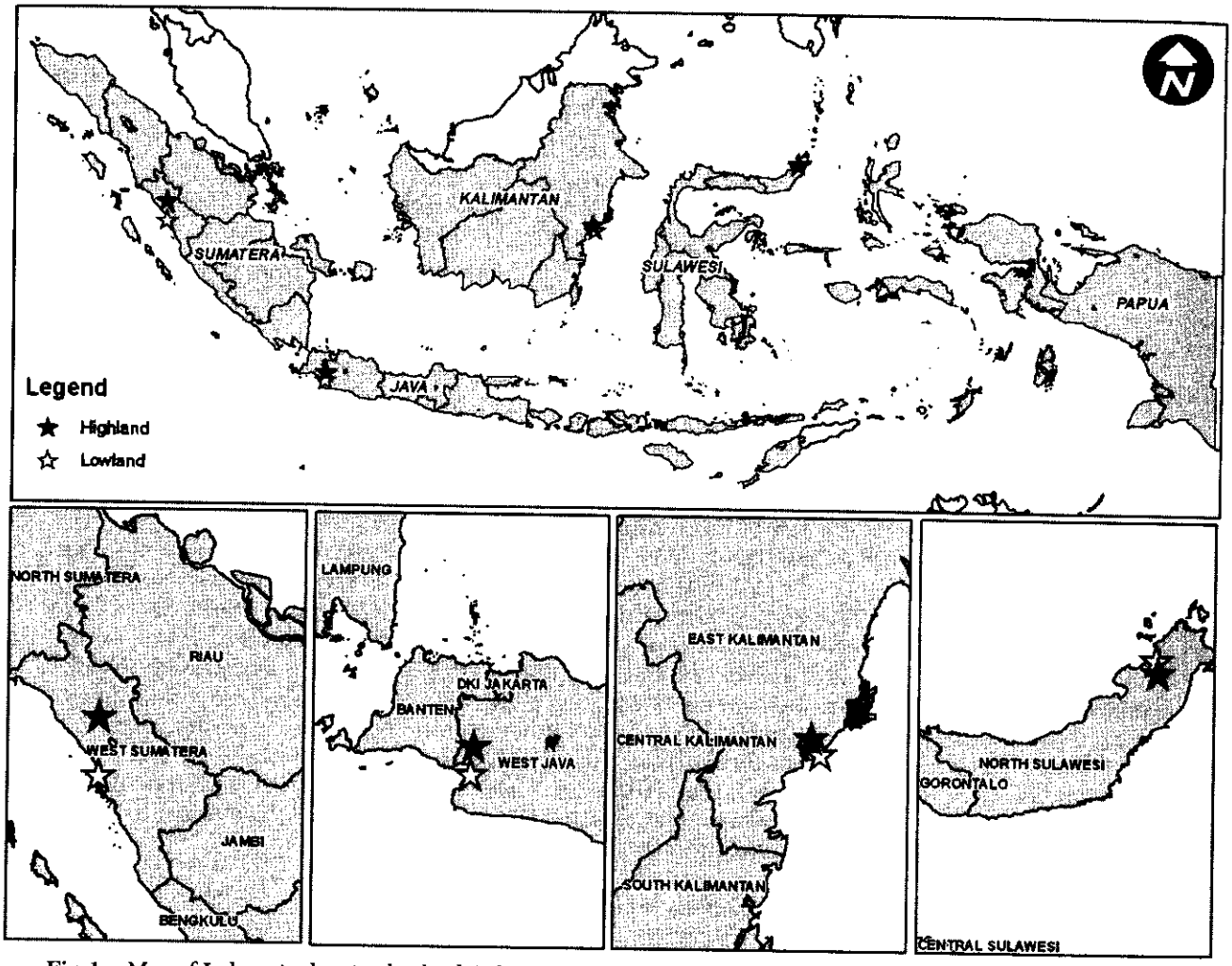

Fig. 1. Map of Indonesia showing lowland (white stars) and highland (black stars) small mammal-trapping locations.

Materials and Methods

Site Description. Fleas were collected as part of larger rodent-borne disease serosurvey focusing on *Rickettsia* spp. Collection sites are shown in Fig. 1. The first collection was conducted in Loji village, Simpenan subdistrict (lowland: 8 m above sea level [asl]; 07°03′12″S/106°32′50″E) and Cihamerang village, Kabandungan subdistrict (highland: 910 m asl; 06°47′31″S/106°34′35″E), Sukabumi District, West Java 21–30 April 2007. The second was conducted 15–21 May 2007 in Air Manis village, Padang District (lowland: 10 m asl; 00°59′09″S/100°21′39″E) and Koto Rantang village, Agam District (highland: 890 m asl; 00°14′45″S/100°21′00″E), West Sumatra. The third field trip was conducted in 22–28 August 2007 in Malalayang village, Manado District (lowland: 35 m asl; 01°26.909′ N/124°49.430′ E) and Kinilow village, Tomohon District (highland: 730 m asl; 01°21.747′ N/124°49.941′ E), North Sulawesi. The final collection was conducted in 23–29 May 2008 in Bukit Bangkirai, Kutai Kartanegara (highland: 110 m asl; 01°01.706′ S/116°51.972′ E) and Manggar Baru, Balikpapan District (lowland: 7 m asl; 01°12.939S/116°58.630′ E), East Kalimantan.

Mammal and Flea Collections. Animals and fleas were collected from lowland (<50 m asl) and highland (>100 m asl) villages on four islands of Indonesia between April 2007 and May 2008. All aspects of animal use were conducted using protocols approved by the NAMRU-2 Institutional Animal Care and Use Committee and the National Institute of Health Research and Development, Indonesian Ministry of Health.

Small mammals were collected using Sherman (H. B. Sherman Traps, Tallahassee, FL) and Tomahawk (Tomahawk Live Trap, Tomahawk, WI) style traps baited with roasted coconut. Tomahawk and Sherman traps were set out in the late afternoon for three nights at each lowland and highland site for a total of six trap nights at each location (four lowland, four highland). Traps were checked the following morning, and those with animals were returned to the processing site. Trapped animals were killed (or sedated) and identified. Ectoparasites were removed from each animal by vigorously brushing to dislodge. After brushing, each animal was examined for any remaining ectoparasites by searching through the pelage with fine forceps. Ectoparasites collected were placed into micro Eppendorf tubes and snap frozen in liquid nitrogen until identified and tested by polymerase chain reaction (PCR) in the laboratory. *Rattus*, *Suncus*, and *Mus* species were killed at the field sites,

and all other species were sedated and then released after sample collection. Fleas were identified as *X. cheopis* or *Nosopsyllus* spp. by using standard taxonomic keys for ectoparasites of commensal rodents (Mahadevan et al. 1969). Flea load (number of fleas on an individual animal) and flea indexes (average number of fleas per animal) were calculated for each mammal species. Voucher specimens are deposited at the United States Naval Medical Research Unit 2 laboratory in Jakarta and will be made available for study in coordination with the Indonesian Ministry of Health.

Pooling Strategy. Fleas were collected and stored at −80°C until evaluated for the presence of rickettsial DNA. For molecular testing, fleas were pooled by individual animal for animals with five or more fleas. Fleas from animals with fewer than five fleas were pooled by site, lowland/highland, and species of small mammal.

DNA Extraction and PCR. DNA was extracted from ectoparasite pools using the QIAmp DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was screened with a *Rickettsia* genus-specific quantitative real-time PCR (qPCR) assay targeting the 17-kDa antigen gene (Jiang et al. 2004). Any positive result was tested by a tick-borne rickettsia-specific qPCR assay (Jiang et al. 2005) and the species-specific *R. felis* and *R. typhi* qPCR assays, which target different regions of *ompB*, as previously described (Jiang et al. 2006).

Extracted DNA was also tested by nested PCR assays that target the 17-kDa antigen gene (*htrA*) of SFG (TZ15/TZ16) and typhus group (RP2/RPID) rickettsiae, as previously described (Blair et al. 2004). Briefly, the broad range primers R17-122 5′-5′-CAG AGT CGT ATG AAC AAA CAA GG-3′ and R17-500 5′- CTT GCC ATT GCC CAT CAG GTT G-3′ were used in the first round PCR with the following cycling conditions: 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s, followed by a final extension at 72°C for 5 min. Separate nested reactions were then performed using 5 μl of first-round product and either a spotted fever genus-specific primer set (TZ15 5′-TTC TCA ATT CGG TAA GGG C-3′ and TZ 16 5′-ATA TTG ACC AGT GCT ATT TC-3′) or a typhus group-specific primer set (RP2 5′-TTC ACG GCA ATA TTG ACC TGT ACT GTT CC-3′ and RPID 5′-CGG TAC ACT TCT TGG TGG CGC AGG AGG T-3′) with the following cycling conditions: 95°C for 5 min, 30 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s, followed by a final extension at 72°C for 5 min. All PCRs were conducted using the GeneAmp PCR kit (Applied Biosystems, Foster City, CA).

Statistics. Fisher exact test was used to compare the number of fleas infected with *Rickettsia* spp. at highland and lowland sites on each island.

Results

Flea Collection. A total of 507 small mammals from four locations in Indonesia was collected and examined for ectoparasites. Fleas were collected from 96 animals (18.97%), and a total of 208 fleas was collected. Ninety-eight percent (98.1%) of fleas collected were *X. cheopis*. *X. cheopis* were collected from *Rattus tanezumi, Rattus exulans, Rattus norvegicus,* and *Suncus murinus* (Table 1). Eight small mammal species (*R. exulans, R. norvegicus, R. tanezumi, Rattus timoanicus, Rattus whiteheadi, Leopoldamys sabanus, Maxomys rajah,* and *Mus musculus*), one shrew species (*S. murinus*), two squirrels (*Sundasciurus lowii* and *Rhinosciurus laticaudatus*), one civet (*Viverra tangalunga*), and one tree shrew (*Tupaia glis*) were collected during this study. *R. tanezumi* had the highest *X. cheopis* load ($n = 20$) among all sites, but *R. exulans* had the highest flea index, 0.7 among all sites.

In West Java, over six trap nights, four rodent species were collected (*R. exulans, R. tanezumi,* and *R. timoanicus*) and the shrew, *S. murinus*. *R. tanezumi* had the highest number of *X. cheopis* collected ($n = 41$; 26 highland, 15 lowland) and the highest flea index, 1.78 (0.88 lowland, 1.73 highland). In West Sumatra, over six trap nights, three rodent species were collected (*R. tanezumi, R. timoanicus,* and *L. sabanus*) and *T. glis* (common tree shrew). *R. tanezumi* had the highest number of *X. cheopis* collected ($n = 90$; 87 highland, three lowland) and the highest flea index, 0.96 (0.06 lowland, 2.02 highland). In North Sulawesi, over six trap nights, six rodent species were collected (*R. tanezumi, R. timoanicus, M. musculus, M. rajah, R. exulans,* and *R. norvegicus*) and the shrew, *S. murinus*. *R. norvegicus* had the highest number of *X. cheopis* collected ($n = 12$; 12 lowland, 0 highland) and the highest flea index, 0.4 (0.4 lowland, 0 highland). In East Kalimantan, over six trap nights, seven rodent species were collected (*R. tanezumi, R. timoanicus, M. rajah, R. exulans, R. whiteheadi, R. norvegicus,* and *L. sabanus*), two squirrel species (*S. lowii* and *R. laticaudatus*); the shrew, *S. murinus;* and the civet, *V. tangalunga*. *R. norvegicus* had the highest number of *X. cheopis* collected ($n = 13$; 13 lowland, 0 highland) and the highest flea index, 0.41 (0.41 lowland, 0 highland).

West Java had the highest overall flea index (0.94), and East Kalimantan had the lowest (0.14). Flea indexes were highest in lowland locations when compared with highland locations at all sites, excluding West Sumatra. Each site varied with respect to number of mammals trapped and number of fleas collected. When all of the *X. cheopis* data from all mammal species is combined for the West Java site, 66 *X. cheopis* were collected for a total flea index of 0.94. *R. tanezumi* had a flea index of 1.28, and *S. murinus* had a flea index of 0.73. At the West Sumatra site, 90 *X. cheopis* were collected for a total flea index of 0.87. *R. tanezumi* had a flea index of 0.96. At the North Sulawesi site, 28 *X. cheopis* were collected with a flea index of 0.15. *R. tanezumi* had a flea index of 0.15, *R. exulans* had a flea index of 0.66, *S. murinus* had a flea index of 0.04, and *R. norvegicus* had a flea index of 0.36. At the East Kalimantan site, 20 *X. cheopis* were collected with a flea index of 0.14. *R. tanezumi* had a flea index of 0.02, *S. murinus* had a flea index of 0.18, and *R. norvegicus* had a flea index of 0.41. Table 1 shows the number of fleas, small mammals, and flea indices for each lowland and highland site at each trapping lo-

Table 1. Mammal species collected, total number of individuals collected, total *Xenopsylla cheopis* collected with flea indexes, and range of number of *X. cheopis* collected during small mammal surveys conducted during 2007–2008 in a) West Sumatra; b) North Sulawesi; c) East Kalimantan; and d), West Java, Indonesia

Mammal species	Lowland				Highland			
	Total no. individuals	Total *X. cheopis*	Flea index	Range	Total no. individuals	Total *X. cheopis*	Flea index	Range
a) West Sumatra								
Rattus tanezumi	51	3	0.06	0–2	43	87	2.02	0–20
Leopoldamys sabanus	0	0	0	0	3	0	0	0
Rattus tiomanicus	4	0	0	0	0	0	0	0
Tupaia glis	3	0	0	0	0	0	0	0
Total	58	3	0.05	0–2	46	87	1.89	0–20
b) North Sulawesi								
Rattus tanezumi	43	10	0.23	0–3	37	2	0.05	0–1
Rattus exulans	1	2	2.00	0–2	2	0	0	0
Suncus murinus	56	2	0.04	0–1	0	0	0	0
Rattus norvegicus	33	12	0.04	0–4	0	0	0	0
Mus musculus	5	0	0	0	2	0	0	0
Rattus tiomanicus	1	0	0	0	0	0	0	0
Maxomys rajah	0	0	0	0	4	0	0	0
Total	139	26	0.19	0–4	45	2	0.04	0–1
c) East Kalimantan								
Rattus tanezumi	12	1	0.08	0–1	28	0	0	0
Rattus exulans	4	0	0	0	11	0	0	0
Suncus murinus	32	6	0.19	0–3	1	0	0	0
Rattus norvegicus	32	13	0.41	0–7	0	0	0	0
Leopoldamys sabanus	0	0	0	0	1	0	0	0
Maxomys rajah	0	0	0	0	1	0	0	0
Rattus laticaudatus	0	0	0	0	5	0	0	0
Rattus tiomanicus	0	0	0	0	8	0	0	0
Rattus whiteheadi	0	0	0	0	6	0	0	0
Sundasciurus lawii	0	0	0	0	5	0	0	0
Viverra tungalunga	0	0	0	0	2	0	0	0
Total	80	20	0.25	0–7	68	0	0	0
d) West Java								
Rattus tanezumi	17	15	0.88	0–3	15	26	1.73	0–9
Rattus exulans	0	0	0	0	2	0	0	0
Suncus murinus	24	17	0.71	0–4	10	8	0.80	0–4
Rattus tiomanicus	1	0	0	0	1	0	0	0
Total	42	32	0.76	0–4	28	34	1.21	0–9

cation. *R. tanezumi* was the most frequently collected mammal at all sites, except the East Kalimantan lowland site, where *R. norvegicus* was the most frequently collected mammal. Total flea load (combined data lowland and highland) was greatest on *R. tanezumi*, except at the Kalimantan site, where the greatest number of fleas were collected on *R. norvegicus*.

***Rickettsia* spp. Testing.** Thirty-seven pools of *X. cheopis* were tested. Six pools were found positive for *Rickettsia* spp. (Table 2); four (10.8%) *R. typhi*, one (2.7%) *R. felis*, and one (2.7%) codetection of *R. felis* and a SFGR. The detection of a tick-borne rickettsia was confirmed to be a member of the SFG of rickettsiae by sequencing regions of the *ompB* (149 bp) and

Table 2. *Rickettsia*-positive *Xenopsylla cheopis* collected during small mammal surveys conducted in 2007–2008 at locations in West Java, West Sumatra, North Sulawesi, and East Kalimantan, Indonesia; data shown derived from *X. cheopis* pools ($n = 37$)

Site	Location	qPCR				Standard PCR		Small mammal species
		htrA[a]	Trick[b]	*R. typhi*[c]	*R. felis*[d]	TG[e]	SFG[f]	
West Java	Highland	+	–	+	–	+	–	*Rattus tanezumi*
West Java	Lowland	+	–	+	–	+	–	*Rattus tanezumi*
West Java	Lowland	+	–	+	–	+	–	*Suncus murinus*
North Sulawesi	Lowland	+	–	–	+	–	–	*Rattus tanezumi*
East Kalimantan	Lowland	+	+	–	+	+	±[g]	*Suncus murinus*
East Kalimantan	Lowland	+	–	+	–	+	–	*Rattus norvegicus*

[a] Target gene htrA conserved rickettsia 17-kD antigen gene.
[b] Target sequence specific for tick-borne SFGR.
[c] Target gene OmpB region specific for *R. typhi*.
[d] Target gene OmpB region specific for *R. felis*.
[e] Primers specific for amplification of OmpB gene fragment of typhus group (TG).
[f] Primers specific for amplification of OmpB gene fragment of SFG.
[g] Visible, but weak band.

ompA (1,328 bp) genes. The amplified genes were found to be 100% identical with *Rickettsia* sp. TwKM01 *ompB* gene (EF219464), and 100% identical with *Rickettsia* spp. TwKM01 *ompA* gene (EF219467) from *rhipicephalus haemaphysaloides*. The detection of *R. felis* was confirmed by sequencing regions of the *r. felis ompB* gene Rf1396 F/Rf1524R (120 bp). The amplified genes were found to be 97% identical with *R. felis ompB* gene (AF. 182279).

There were no significant differences between rickettsial infections in fleas collected from small mammals from highland or lowland sites of all islands (Fisher exact test West Java $P = 0.37$; North Sulawesi $P = 0.93$; East Kalimantan $P = 0.82$). No *Nosopsyllus* spp. tested positive for *Rickettsia* spp.

Discussion

To determine the identity of rickettsial agents infecting fleas from small mammals in Indonesia, we assessed 37 pools of fleas collected from small mammals on four islands in Indonesia. We report molecular detection and identification of *R. typhi*, *R. felis*, and a SFGR associated with oriental rat fleas (*X. cheopis*) collected from small mammals on four islands in Indonesia. Our results report that *R. typhi* is present in a known flea vector, *X. cheopis*, of murine typhus in Java (Corwin et al. 1997, Richards et al. 1997, Jiang et al. 2006) and report the first evidence of infection in fleas found on Kalimantan, Indonesia. The causative agent of murine typhus is *R. typhi* and the vector for *R. typhi* in Indonesia is the Asiatic rat flea (*X. cheopis*). Humans can become infected with *R. typhi* when an infected flea contaminates the feeding site, skin abrasions, or by the bite of infected fleas (Azad and Traub 1985). Murine typhus is endemic to Indonesia and has been found on the islands of Java, Sumatra, Bali, and Irian Jaya (Richards et al. 2002).

R. felis, the agent that causes flea-borne spotted fever, has been shown to infect fleas of peridomestic rodents and fleas other than the primary vector, *Ctenocephalides felis*, in Java, Indonesia (Azad et al. 1997, Parola et al. 1998, Richards et al. 2003, Jiang et al. 2006). *R. felis* was first shown to infect Indonesian *X. cheopis* collected in Java in 1994 (Jiang et al. 2006). Although *R. felis* is phylogenetically more closely related to the SFGR than the typhus group, it shares antigens with *R. typhi*, a typhus group rickettsia, and produces similar symptoms (Azad et al. 1997, Higgins et al. 1996).

In the current study, *R. felis* was detected in *X. cheopis* on small mammals collected from North Sulawesi and East Kalimantan. This is the first report of *R. felis* in *X. cheopis* in parts of Indonesia other than on the island of Java. *R. felis* was detected from flea pools taken from *R. tanezumi* in Sulawesi and the shrew, *S. murinus*, in Kalimantan. Both of these fleas were taken from animals collected in/around houses at the study site rather than the forested areas. *R. tanezumi* and *S. murinus* were the predominant species collected in the lowland sites of Java, Sumatra, and North Sulawesi, with *R. tanezumi* only predominating in the highland sites. *R. norvegicus* and *S. murinus* were the predominant species in the Kalimantan lowland site, with *R. tanezumi* and *R. exulans* predominate in the highland site. *R. tanezumi* and *S. murinus* appear to be the primary host for *R. typhi*- and *R. felis*-infected *X. cheopis*. All fleas that tested positive for *R. typhi*, *R. felis*, or a SFGR were collected in the lowland sites, except for one *R. typhi*-infected *X. cheopis* found at the highland site in West Java on *R. tanezumi*.

In one pool of *X. cheopis* collected from East Kalimantan, both *R. felis* and a SFGR were detected. The SFGR are composed of >20 antigenically related rickettsial species (Fournier and Raoult 2009). SFGR have been reported in Southeast Asia, and evidence for their presence in Indonesia is accumulating (Richards et al. 1997).

The occurrence of rickettsial agents in arthropods found on various small mammal species suggests the pathogens may have a widespread distribution throughout Indonesia. The rat reservoir not only serves as a host for the flea vector, but also makes rickettsiae available in the blood for fleas, which may transmit rickettisae back to a rat host during subsequent feeding (Azad 1990). In urban environments of Indonesia, *R. rattus* and *R. norvegicus* rats are likely to be the main hosts harboring *R. typhi*-infected *X. cheopis* (Jiang et al. 2006). Our study found that *R. tanezemi* is a host for *X. cheopis* infected with *R. typhi* and *R. felis*. We also report finding *R. typhi* and a codetection of *R. felis* and a SFGR from a pool of *X. cheopis* found on *S. murinus*. The codetection may be a common occurrence in fleas from this region or it may be the result of a flea infected with *R. felis* feeding on a small mammal infected with a SFGR, resulting in detection of two agents, one from the flea and one from the infected blood consumed by the flea.

Our findings reveal that levels of rickettsial infections in fleas from small mammals collected in lowland areas were not significantly different from levels found in highland, suggesting that hosts and vectors are widespread and the risk of infection is similar wherever host and vectors are found together. We found evidence of *Rickettsia* spp. pathogens, the hosts, and the vector in and around human habitation in four locations in Indonesia and a new report of *R. felis* in *X. cheopis* iu parts of Indonesia other than on the island of Java. These findings merit further investigation to better understand the relationship between *Rickettsia* spp., *X. cheopis*, and the transmission dynamics between flea and small mammal host. Flea-borne rickettsial infections pose a threat to human populations in Indonesia and should be considered by clinicians upon the presentation of febrile disease among patients from endemic areas.

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